

Amendment and Response

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Serial No.: 09/180,340

Confirmation No.: 6674

Filed: 20 August 1999

For: STABLE RECOMBINANT YEASTS FOR FERMENTING XYLOSE TO ETHANOL**Amendments to the Claims**

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

Listing of Claims

1-13. (canceled)

14. (currently amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(a) transforming the cells with [[a]] at least one replicative and integrative plasmid comprising each of the following: [[an]] a yeast autonomous replicating sequence, exogenous DNA, and a first selection marker; and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

15. (original) The method of claim 14, wherein the plasmid DNA also includes a second selection marker for selecting cells which include the plasmid.

16. (original) The method of claim 14 wherein the cells are yeast or eukaryotic cells, and wherein the method further includes the step of repeatedly replicating the progeny cells from step (b) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

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17. (original) The method of claim 16 wherein the cells are yeast cells and the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, which also serve as the first selection marker.

18. (currently amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(i) transforming yeast cells with [[a]] at least one replicative and integrative plasmid comprising each of the following: [[an]] a yeast autonomous replicating sequence, exogenous DNA comprising genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, wherein the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, and a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly replicating the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA, wherein the progeny cells ferment xylose to ethanol; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA, wherein fermentation activity of the yeast cells of step (iii) is not decreased after culture in non-selective medium for greater than 40 generations.

19. (original) Yeast cells produced by the method of claim 18.

20. (canceled)

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21. (currently amended) The yeast cells of claim 20 ~~19~~, wherein said genes are fused to non-glucose-inhibited promoters which do not require xylose for induction, and wherein the yeast cells ferment glucose and xylose simultaneously to ethanol.

22-27. (canceled)

28. (currently amended) A plasmid vector comprising a functional yeast autonomous replicating sequence and an exogenous DNA comprising a first selection marker, the exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further including a second section selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell.

29. (previously presented) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of the target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol.

30. (currently amended) A method for producing cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising each of the following: ~~[[an]]~~ a yeast autonomous replicating sequence and ~~containing~~ the exogenous DNA, to produce multiple generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce

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progeny cells having multiple integrated copies of the exogenous DNA.

31. (canceled)

32. (previously presented) The method of claim 14 wherein the cells are yeast.

33. (previously presented) The method of claim 30 wherein the cells are yeast.

34. (previously presented) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell.